bio-protocol Preprint

Not Peer Reviewed
Ask a question online

Preparation of samples for Ribo-seq

MS Maxim S. Svetlov DW Daniel N. Wilson AM Alexander S. Mankin

Updated date: Jun 25, 2022

An abbreviated version of this protocol was published in Nature Communications in May 2021

Context-specific action of macrolide antibiotics on the eukaryotic ribosome

DOI: 10.1038/s41467-021-23068-1

Detailed protocol

1. We grew *S. cerevisiae* cells exponentially at 30 °C in two 1 L flasks containing 200 mL of YPD medium.
2. When the culture density reached A_{600} ~ 0.6, the cells were treated with 1.5 mg/mL telithromycin (Comment: this step can be skipped depending on your experiment).
3. Treated cells were collected by rapid filtration through Express Plus® Membrane filter (Millipore). Cells were flesh frozen in liquid nitrogen Comment: the cells can be stored in -80°C frige for at least several weeks untill the next step).
4. The cells were lysed using Mixer Mill MM400 (Retsch) with 300 µL of lysis buffer (10 mM Hepes-KOH, pH 7.5, 50 mM KOAc, 10 mM NH_4Cl, 2 mM DTT, 5 mM $Mg(OAc)_2$) without addition of cycloheximide (Comment: in our case cells were treated with telithromycin).
5. Lysates were cleared by centrifugation (10 min 20,000 × *g* at 4 °C) and supernatants were treated with 30 U/A_{260} of RNaseI (Ambion) at 4 °C for 5 min (Comment: for each new RNaseI preparation I recommend titrating amount of the RNase and time of treatment and analyze conversion of polysomes into the 80S ribosomes by sucrose gradient).
6. Three hundred microliter of digested lysates were loaded onto 500 µL of 25% (w/v) sucrose cushion in buffer 20 mM Tris-HCl, pH 8.0, 140 mM KCl, 10 mM $MgCl_2$ supplemented with 1× Complete protease inhibitor. Ribosomes were pelleted by centrifugation in a TLA100.2 rotor at 90,000 rpm (350,000 g), 4 °C for 1 h.
7. Pellets were resuspended in 500 µL of 1% SDS and ribosome-protected mRNA fragments were isolated by standard phenol-chloroform extraction followed by ethanol precipitation.
8. RNA pellets were resuspended in 20 µL of TE buffer and half of it was run in 15% polyacrylamide denaturing gel Comment: another 10 µL of the isolated RNA can be stored at -80°C).
9. RNA footprints ranging in size between ~20 and ~35 nt were excised and eluted from the gel.
10. RNA footprints were converted to sequencing libraries as described by Becker et al.[89] (for replicate 1) or McGlincy & Ingolia[88] (for replicate 2). (Comment: I recommend using McGlincy & Ingolia protocol (Methods, 126, 112-129 (2017)).
11. The libraries were sequenced at the NUSeq Core (Northwestern University) on Illumina HiSeq 4000 platform.

Related files

Becker_2013.pdf

McGlincy_2017.pdf

How to cite: (Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Svetlov, M. , Wilson, D. and Mankin, A. (2022). Preparation of samples for Ribo-seq. Bio-protocol Preprint. bio-protocol.org/prep1742.
2. Svetlov, M. S., Koller, T. O., Meydan, S., Shankar, V., Klepacki, D., Polacek, N., Guydosh, N. R., Vázquez-Laslop, N., Wilson, D. N. and Mankin, A. S.(2021). Context-specific action of macrolide antibiotics on the eukaryotic ribosome. Nature Communications 0(0). DOI: 10.1038/s41467-021-23068-1